AD NUMBER

AD838753

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Critical Technology; JUN 1968. Other requests shall be referred to Dept. of the Army, Fort Detrick, MD 21701.

AUTHORITY

BDRL D/A ltr, 29 Sep 1971

AD

TECHNICAL MANUSCRIPT 467

A RAPID SENSITIVE ASSAY FOR STAPHYLOCOCCAL ENTEROTOXIN AND A COMPARISON OF SEROLOGICAL METHODS

Sidney J. Silverman
Allen R. Knott
Mary B. Howard

JUNE 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

DDC AVAILABILITY NOTICES

Qualified requesters may obtain copies of this publication from DDC.

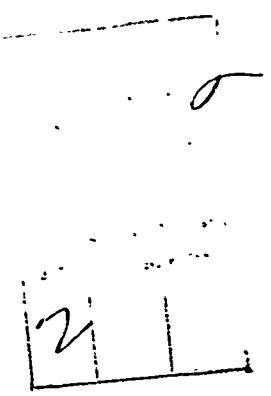
Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.



DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 467

A RAPID SENSITIVE ASSAY FOR STAPHYLOCOCCAL ENTEROTOXIN AND A COMPARISON OF SEROLOGICAL METHODS

Sidney J. Silverman

Alan R. Knott

Mary B. Howard

Medical Bacteriology Division
BIOLOGICAL SCIENCES LABORATORY

Project 1B522301A059

June 1968

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Reversed passive hemagglutination was used to assay enterotoxin in culture filtrates and in food samples. Using cells preserved with either formaldehyde or pyruvic aldehyde, which were tanned and then sensitized with antitoxin globulin, as little as 0.0007 μg of enterotoxin was detectable. The results of hemagglutination tests compared well with those obtained by quantitative precipitin tests or by immunodiffusion, but hemagglutination was 50 to 100 times more sensitive than the immunodiffusion technique. In addition, results of the hemagglutination test are available within a few hours, and neither elimination of interfering proteins from food extracts nor concentration of the sample, both of which are necessary for immunodiffusion, is required for this procedure.

I. INTRODUCTION

The purification of staphylococcal enterotoxin has resulted in the recognition of at least four serological types[1-3] and the development of serological tests for detection and assay. Methods for immunodiffusion in agar, quantitative precipitin assays, hemagglutination inhibition, and immunofluorescence have been described for this purpose.[4-10] Measurement of enterotoxin in food samples by immunofluorescence, a micro-Ouchterlony technique, and the single diffusion technique of Oudin have been described, as have the procedures necessary to eliminate nonspecific proteins and salts that influence the results.[11-16] Hemagglutination inhibition, passive hemagglutination, and the quantitative precipitin test have not been used with food samples. It seemed that these procedures would be less influenced by the presence of the nonspecific substances that affect immunodiffusion; thus, the lengthy procedures described for processing food extracts could be eliminated.[11,14] In addition, hemagglutination should permit the detection of smaller amounts of enterotoxin than do the other methods without the necessity of concentrating the food extracts.

Hemagglutination of sheep erythrocytes, to which enterotoxin B was attached by means of bis-diazotized benzidine, has been used in our laboratory for several years to estimate serum antibody titers. Johnson, Hall, and Simon[7] used such cells in an inhibition test to measure enterotoxin in culture filtrates. Cook[17] described a procedure that he termed "reversed passive hemagglutination" for the assay of tetanus toxin. Antitoxin globulin attached directly to erythrocytes permitted direct measurement of toxin by hemagglutination rather than following a two-step procedure such as hemagglutination inhibition. Sinitsyn[18] used a similar technique for detecting botulinal toxin. We describe here the use of "reversed passive hemagglutination" for the assay of enterotoxin B in culture filtrates and in food samples to which toxin was added. The report also compares the data obtained by this procedure with those obtained by immunodiffusion and the quantitative precipitin technique.

II. MATERIALS AND METHODS

Formalin-preserved sheep erythrocytes* (SRBC) were washed once with 0.038 M $NaHSO_3$ in 0.85% saline and then twice with saline. We observed previously[19] that formaldehyde (HCHO) decreased the serological activity of enterotoxin B; therefore, the initial washing with $NaHSO_3$ was included to neutralize free aldehyde. Fresh SRBC collected in an equal volume of modified Alsever's solution[20] were treated with pyruvic aldehyde (CH_3COCHO) as described by Ling.[21]

* Obtained from Difco Laboratories, Detroit, Michigan.

Anti-enterotoxin globulin was prepared from rabbit antiserum by saturating with $(NH_4)_2SO_4$ to 50%. The precipitate was washed several times with 50% saturated $(NH_4)_2SO_4$, dialyzed against 0.15 M borate buffered saline (pH 8.4) until free of $SO_4^=$ ions, and then freeze-dried and stored at 4 C. Approximately 90% of the antibody was recovered. Rabbits were immunized as described previously.[9] Recent data indicate that initial injections of toxoid in Freund's adjuvant into the foot pad, followed after 3 to 4 weeks by intravenous injections of alum-adsorbed toxoid and then alum-adsorbed toxin, would produce high-titered antitoxin in a shorter time interval than with the original procedure.

Cultures of Staphylococcus aureus strain S6 grown for 8 to 24 hours in 2.0% protein hydrolyzate powder* were filtered through ultrafine sintered glass filters to provide sterile filtrates.[22] Before immunodiffusion assays, they were dialyzed against 0.02 M phosphate buffered saline (PBS), pH 7.3. Purified enterotoxin B was obtained from Dr. E.J. Schantz, Fort Detrick. Frozen cream, custard, meat, and tuna pies were thawed and the pastry portion was discarded. The fillings were homogenized in a Waring Blendor with an equal amount of distilled water (v/v), and culture filtrate containing known quantities of enterotoxin B was added. The homogenates were further diluted with equal volumes of 0.02 M PBS, pH 6.4, and heated at 50 C for 15 minutes. After standing for 45 minutes at room temperature, they were centrifuged at about 15,000 x g for 20 minutes in a Sorvall SS-1 centrifuge at 4 C. The clear liquid portion between the sediment and the top fat layer was removed with a syringe holding an 18-gauge needle and then filtered through a single layer of paper towel.**[14] Cheese and milk were prepared essentially as described by Read et al.[15,16] The culture filtrate containing enterotoxin was added to the homogenized cheese or directly to the milk before separation of the whey. For hemagglutination (HA) tests, serial dilutions of the food extracts were prepared in PBS, pH 7.3, containing 1.0% normal rabbit serum.

SRBC were tanned and sensitized as described by Boyden.[23] Antitoxin globulin diluted to contain 50.0 µg antibody nitrogen per ml was used to sensitize a 2.5% suspension of SRBC or a 1.0% suspension of SRBC for use in microtiter tests. In the tube tests, 0.5-ml amounts of serial 1:2 dilutions of the enterotoxin samples were distributed into a series of tubes, and 0.05 ml of SRBC treated with globulin antitoxin was added. After standing at room temperature for 2 hours, the degree of agglutination was determined from the pattern of sedimented cells. For the microtiter test, serial dilutions of the enterotoxin sample were prepared in the wells of

* Mead Johnson & Co., Evansville, Indiana.
** Scott Wipers 590, Scott Paper Co., Chester, Pa.

plastic (hes using microtiter loops; 0.025 ml of 1.0% SRBC was added to 0.025 ml of dilution, and the tests were read after 2 hours at room temperature. Toxin concentrations in the samples were calculated by multiplying the reciprocal of the greatest dilution of sample that reacted by the smallest amount of purified standard enterotoxin that gave a positive result in the control test. With the antiserum used for these studies, 0.0015 μg of enterotoxin B per ml was generally the smallest concentration that caused distinct hemagglutination.

The quantitative precipitin tests were performed as described previously.[5] After incubation at 37 C for 3 to 4 hours and at 4 C for 4 days, the precipitates were washed and dissolved in 0.25 N acetic acid, and the optical density was determined at $\lambda = 277$ mμ. The concentration of enterotoxin in the sample was determined from a standard curve relating optical density of the dissolved precipitate to the concentration of enterotoxin reacting with the antibody. The antiserum supernatant fluid was tested for excess antigen and excess antibody by double immunodiffusion. In samples with more than 10.0 μg of enterotoxin per test, incubation at 37 C for 6 hours appeared to be sufficient for complete precipitation, so that assay of culture filtrates, for example, could be completed easily within 8 hours.

Immunodiffusion tests were performed by layering the sample on a mixture of equal volumes of 1.0% agar and a suitable concentration of antitoxin. The tubes were placed in a 30 C water bath. The distance of the leading margin of the precipitate band from the agar meniscus was measured with a cathetometer three times at approximately 24-hour intervals. The distance of band movement was plotted against the square root of time to obtain the slope (k). The concentration of toxin was determined from a standard curve relating the value of k to the concentration of enterotoxin. The standard curve was prepared from the results obtained with known concentrations of purified enterotoxin B diluted in brain heart infusion broth diluted 1:1 with 0.02 M PBS, pH 7.3.[9]

III. RESULTS

Table 1 shows a portion of the data obtained when culture filtrates were analyzed for enterotoxin by the procedures studied. In most cases exceptionally good agreement was obtained with the various techniques. Microtiter results (not shown), obtained after the filtrates were stored at -20 C for about 3 months, corresponded well with the results of the tests performed earlier by the other procedures. In general, titers obtained by the microtiter technique were twofold (one dilution) less than those obtained with the tube test. No cross reactions were obtained with antigens in culture filtrates formed by either staphylococci that produced type A enterotoxin or nontoxigenic strains.

TABLE 1. COMPARISON OF SEROLOGICAL TESTS FOR DETECTION OF ENTEROTOXIN B IN STAPHYLOCOCCAL CULTURE FILTRATES

Filtrate	Enterotoxin B, μg/ml			
	Immunodiffusion (Ouchterlony)	Hemagglutination[a] HCHO	Hemagglutination[a] CH_3COCHO	QPT[b]
WZ14	100.0	99.2	198.0	93.7
WZ19	26.0	19.8	19.8	28.5
WZ27	39.0	19.8	19.8	39.7
Old P2	49.0	19.8	39.7	46.0
WZ21	59.0	39.7	39.7	55.5
Old P3	57.0	39.7	39.7	48.6
P1	<4.0	4.9	4.9	11.0
WZ7	54.0	99.2	99.2	55.9
Old P1	52.0	39.7	39.7	46.0
17-5	40.0	19.8	39.7	36.0

a. Hemagglutination with SRBC preserved with HCHO or CH_3COCHO, tanned, and then sensitized with antitoxin globulin.
b. QPT = quantitative precipitin test.

Assays for enterotoxin added to various food samples are presented in Tables 2 and 3. The data in Table 2 were obtained with frozen foods that had been thawed but not cooked before homogenization and the addition of enterotoxin; Table 3 shows results obtained with cooked products. It was difficult to clarify the liquid separated from the homogenates of the uncooked tuna or custard pies, so it was not possible to determine the results of the precipitin test of the custard by the procedure used. Again, good agreement was obtained with the different tests. Because the HA tests are semiquantitative, only a difference greater than fourfold (two tubes) was considered significant. No HA reactions were obtained when the samples were incubated with tanned unsensitized cells, and food homogenates did not react with sensitized SRBC in the absence of enterotoxin.

TABLE 2. COMPARISON OF SEROLOGICAL TESTS FOR DETERMINATION OF ENTEROTOXIN IN FOOD SAMPLES

Food[a]	Enterotoxin B Content, µg/ml		
	Hemagglutination (HCHO-SREC)	QPT[b]	Immunodiffusion (Oudin)
Tuna pie	3.8	7.1	5.5
Cheddar cheese	7.7	6.8	7.7
Milk	7.7	4.9	4.9
Beef pie	7.7	4.9	5.1
Coconut cream pie	3.8	[c]	5.5

a. Frozen pies were thawed but not cooked. All food homogenized with an equal volume of distilled water and one part staphylococcal culture filtrate containing 31.5 µg enterotoxin per ml added to four parts of homogenate so that each food sample contained 6.3 µg enterotoxin B per ml.
b. QPT = quantitative precipitin test.
c. Acetic acid solution of washed specific precipitate was cloudy, possibly due to starch in the filling.

TABLE 3. ASSAY OF ENTEROTOXIN B IN COOKED FOOD HOMOGENATES[a]

Food	Enterotoxin Added, µg/ml	Enterotoxin Measured, µg/ml			
		Hemagglutination[b]		QPT[c]	Immuno-diffusion
		HCHO	CH_3COCHO		
Cheese	10.0	5.0	10.0	6.9	8.2
	0.1	0.1	0.2		
	0.01	0.01	0.02		
Chicken pie	10.0	10.0	10.0	7.1	6.7
	0.1	0.1	0.2		
	0.01	0.01	0.02		
Banana cream pie	10.0	5.0	10.0	7.4	8.2
	0.1	0.1	0.2		
	0.01	0.01	0.01		
Tuna pie	10.0	5.0	10.0	8.9	5.8
	0.1	0.1	0.2		
	0.01	0.01	0.02		
Control (enterotoxin B)	10.0			6.1	8.8

a. The enterotoxin was added to the samples after the frozen foods had been cooked according to the manufacturer's directions.
b. Hemagglutination tests with SRBC preserved with HCHO or CH_3COCHC, tanned, and then sensitized with antitoxin globulin.
c. QPT = quantitative precipitin tests.

IV. DISCUSSION

The data presented indicate that reliable, simple serological methods for enterotoxin assay are available. HA is a more sensitive procedure for detection of small amounts than are the immunodiffusion procedures described by others.[8,13,14] The mean end point for about 30 HA tests with purified enterotoxin was 0.0015 µg per .' (or 0.0007 µg, the actual amount in the 0.5-ml volume of the test). Oakman and Bennett[11] reported a sensitivity of 0.1 µg for the micro-immunodiffusion technique, while Hall et al.[13] detected 0.05 µg by the Oakley immunodiffusion procedure.[24]

The sensitivity of HA is a disadvantage when relatively high concentrations of enterotoxin are present, for example, in culture filtrates. The long series of dilutions necessary to obtain an end point introduces considerable error. Furthermore, in calculating the concentration, the inordinately large value of the dilution factor can introduce considerable error. On the other hand, the sensitivity and simplicity of the procedure are of extreme value for estimating small concentrations of toxin such as might be found in food samples obtained from a food poisoning event. When materials containing higher concentrations of enterotoxin (e.g. culture filtrates) are to be assayed, greater accuracy can be obtained by preparing the initial dilutions in volumetric flasks using analytical techniques and precautions.

No significant differences were observed in tests performed with SRBC preserved with the two aldehydes. HCHO seems to be the reagent of choice because these cells appeared to store better at 4 C than did cells treated with CH_3COCHO.

Occasional HA tests gave results that were in disagreement with those obtained by the other procedures. Examination of the data indicated that the cause was the variation of the end point for the standard toxin from the mean of 0.0015 µg/ml. With some titrations the smallest amount of purified toxin that caused agglutination was only one-tenth of this value. However, the results obtained with the unknown samples were in agreement with those obtained by immunodiffusion or by precipitation when, for the calculation of the toxin in unknown samples, the mean value for sensitivity of the globulin (0.0015 µg) was used rather than the actual value obtained with the standard enterotoxin sample tested simultaneously with the unknowns. Furthermore, if the smallest amount of toxin in the unknown that produced HA was calculated on the basis of toxin concentration as determined by other procedures (e.g. immunodiffusion), it was close to the value 0.0015 µg/ml, which was the end point for the antitoxin globulin used in these experiments. The explanation for this disagreement can probably be traced to deterioration of the standard toxin preparations. Stock toxin solutions containing 1.0 µg/ml were dispensed in small volumes

and stored at -20 C. Occasional vials appear to give aberrant results. Precautionary steps have been introduced to avoid this. Standard toxins are now stored in more concentrated solutions (25 or 50 ug/ml), and the actual concentration is confirmed by immunodiffusion or precipitin tests. The results of any HA tests with the standard solution that are significantly different from the mean value of 0.0015 µg/ml are regarded as unreliable and the titration is repeated with a fresh standard solution.

The agreement obtained with the three tests indicates that they are all reliable procedures. The choice of test to be used for investigative purposes is, in part, dependent upon the speed and accuracy required. When high concentrations of enterotoxin are to be assayed, quantitative precipitin assay appears to be the procedure of choice. Accurate assays have been obtained within an 8-hour day when a serum containing sufficient antibody for rapid precipitation was used. In this case, the samples were kept at 37 C for 4 to 6 hours before the precipitates were washed, dissolved, and assayed. The standard curve was prepared under identical conditions. Immunodiffusion by the Oudin technique has also been useful for samples containing from 5 to 200 µg of toxin per ml. However, longer incubation is necessary and, in the case of such samples as food extracts, elimination of interfering salts and proteins is necessary. For low concentrations, "reversed passive hemagglutination" is the method of choice. A screening technique for preliminary assay could be devised by sensitizing the red blood cells with antitoxin globulin prepared against the various serological types of enterotoxin as these become available.

In ancillary studies not detailed here, we observed that burro globulin gave less satisfactory results than did rabbit antitoxin globulin; therefore, the latter was used in these studies. The concentration of globulin required for optimal fixation onto tanned SRBC was determined by titrating known quantities of toxin with SRBC treated with various concentrations of globulin. The dilution selected, 1:8, was slightly in excess of the minimal concentration required for maximal agglutination with the antiserum globulin used. Bis-diazotized benzidine, already used to couple enterotoxin to SRBC, was also investigated, but we failed to attain optimal conditions for conjugation of SRBC with globulin with bis-diazotized benzidine.

Preliminary studies on the storage of tanned, HCHO- or CH_3COCHO-preserved SRBC sensitized with antitoxin indicated that SRBC preserved with HCHO were satisfactory for at least 4 months; the CH_3COCHO-preserved cells became unsatisfactory in about 1 month. The cell suspensions were stored at 4 or -20 C and tested at intervals. A few studies indicated that tanned, sensitized HCHO-treated cells were also satisfactory when freeze-dried and reconstituted as described by Cook.[17]

HCHO-treated SRBC sensitized with antitoxin were tested against several lots of purified enterotoxin and a number of staphylococcal culture filtrates. The end points of these titrations (0.001 μg to 0.0002 μg) were not significantly different. They do indicate however, the variations inherent in a test based on serial dilutions.

"Reversed passive HA" is a sensitive procedure for the detection of staphylococcal enterotoxin B. With the reagents and procedures currently in use, 0.0015 μg/ml or the absolute amount of 0.0007 μg of toxin can be detected. The method is more rapid than the immunodiffusion procedures currently in use, and it does not require prior concentration of the sample because of its sensitivity. Other proteins and constituents present in filtrates or food extracts do not appear to interfere. However, the sample should be near neutrality in reaction and, if necessary, should be dialyzed to remove excess salts.

LITERATURE CITED

1. Bergdoll, M.S.; Borja, C.R.; Avena, R.M. 1965. Identification of a new enterotoxin as enterotoxin C. J. Bacteriol. 90:1481-1485.

2. Casman, E.P.; Bergdoll, M.S.; Robinson, J. 1963. Designation of staphylococcal enterotoxins. J. Bacteriol. 85:715-716.

3. Casman, E.P.; Bennett, R.W.; Dorsey, A.E.; Issa, J.A. 1967. Identification of a fourth staphylococcal enterotoxin, enterotoxin D. J. Bacteriol. 94:1875-1882.

4. Donnelly, C.B.; Leslie, J.E.; Black, L.A.; Lewis, K.H. 1967. Serological identification of enterotoxigenic staphylococci from cheese. Appl. Microbiol. 15:1382-1387.

5. Dalidowicz, J.E.; Silverman, S.J.; Schantz, E.J.; Stefanye, D.; Spero, L. 1966. Chemical and biological properties of reduced and alkylated staphylococcal enterotoxin B. Biochemistry 5:2375-2381.

6. Genigeorgis, C.; Sadler, W.W. 1966. Immunofluorescent detection of staphylococcal enterotoxin B: I. Detection in culture media. J. Food Sci. 31:441-449.

7. Johnson, H.M.; Hall, H.E.; Simon, M. 1967. Enterotoxin B: Serological assay in cultures by passive hemagglutination. Appl. Microbiol. 15:815-818.

8. Morse, S.A.; Mah, R.A. 1967. Microtiter hemagglutination inhibition assay for staphylococcal enterotoxin B. Appl. Microbiol. 15:58-61.

9. Silverman, S.J. 1963. Serological assay of culture filtrates for Staphylococcus enterotoxin. J. Bacteriol. 85:955-956.

10. Weirether, F.J.; Lewis, E.E.; Rosenwald, A.J.; Lincoln, R.E. 1966. Rapid quantitative serological assay of staphylococcal enterotoxin B. Appl. Microbiol. 14:284-291.

11. Casman, E.P.; Bennett, R.W. 1965. Detection of staphylococcal enterotoxin in food. Appl. Microbiol. 13:181-189.

12. Genigeorgis, C.; Sadler, W.W. 1966. Immunofluorescent detection of enterotoxin B: II. Detection in foods. J. Food Sci. 31:605-609.

13. Hall, H.E.; Angelotti, R.; Lewis, K.H. 1963. Quantitative detection of staphylococcal enterotoxin B in food by gel-diffusion methods. Public Health Rep. 78:1089-1098.

14. Hall, H.E.; Angelotti, R.; Lewis, K.H. 1965. Detection of staphylococcal enterotoxin in food. Health Lab. Sci. 2:179-191.

15. Read, R.B., Jr.; Pritchard, W.L.; Bradshaw, J.; Black, L.A. 1965. In vitro assay of staphylococcal enterotoxins A and B from milk. J. Dairy Sci. 48:411-421.

16. Read, R.B., Jr.; Pritchard, W.L.; Bradshaw, J.; Black, L.A. 1965. Assay of staphylococcal enterotoxin from cheese. J. Dairy Sci. 48:420-424.

17. Cook, R.J. 1965. Reversed passive hemagglutination systems for the estimation of tetanus toxins and antitoxins. Immunology 8:74-80.

18. Sinitsyn, V.A. 1960. Use of the indirect hemagglutination reaction in detection of botulinum toxins: Report I. Zh. Mikrobiol. Epidemiol. i Immunobiol. 31:22-27.

19. Silverman, S.J.; Schantz, E.J.; Espeseth, D.A.; Roessler, W.G. 1966. Effect of formalin on the immunochemical and biological activity of staphylococcal enterotoxin. Bacteriol. Proc. p. 43.

20. Bukantz, S.C.; Rein, C.R.; Kent, J.R. 1946. Studies in complement-fixation: II. Preservation of sheep's blood in citrate dextrose mixtures (modified Alsever's solution) for use in the complement-fixation reaction. J. Lab. Clin. Med. 31:394-399.

21. Ling, N.R. 1961. The attachment of proteins to aldehyde-tanned cells. Brit. J. Haematol. 7:299-302.

22. Friedman, M.E. 1966. Inhibition of staphylococcal enterotoxin B formation in broth cultures. J. Bacteriol. 92:277-278.

23. Boyden, S.V. 1951. The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. J. Exp. Med. 93:107-120.

24. Oakley, C.L.; Fulthorpe, A.J. 1953. Antigenic analysis by diffusion. J. Pathol. Bacteriol. 65:49-60.

Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)

Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a. REPORT SECURITY CLASSIFICATION

Unclassified

2b. GROUP

3. REPORT TITLE

A RAPID SENSITIVE ASSAY FOR STAPHYLOCOCCAL ENTEROTOXIN AND A COMPARISON OF SEROLOGICAL METHODS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)

Sidney J. Silverman
Allen R. Knott
Mary B. Howard

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
June 1968	15	24

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B522301A059	Technical Manuscript 467
c.	9b. OTHER REPORT NOS (Any other numbers that may be assigned this report)
d.	

10. DISTRIBUTION STATEMENT

Qualified requesters may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Department of the Army Fort Detrick, Frederick, Maryland, 21701

13. ABSTRACT

Reversed passive hemagglutination was used to assay enterotoxin in culture filtrates and in food samples. Using cells preserved with either formaldehyde or pyruvic aldehyde, which were tanned and then sensitized with antitoxin globulin, as little as 0.0007 µg of enterotoxin was detectable. The results of hemagglutination tests compared well with those obtained by quantitative precipitin tests or by immunodiffusion, but hemagglutination was 50 to 100 times more sensitive than the immunodiffusion technique. In addition, results of the hemagglutination test are available within a few hours, and neither elimination of interfering proteins from food extracts nor concentration of the sample, both of which are necessary for immunodiffusion, is required for this procedure.

14. Key Words

*Staphylococcal enterotoxin
*Assay
Immunodiffusion
Hemagglutination

DD FORM 1 NOV 68 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification